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Endogenous Sodium-Potassium-Chloride Cotransport Inhibitor in Congestive Heart Failure

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Objectives. **This study sought to evaluate the relation, if any, between fluid overload in congestive heart failure (CHF) and a newly discovered endogenous natriuretic factor acting like loop diuretic drugs: cotransport inhibitory factor (CIF).**

Background. **The humoral mechanisms regulating volume overload in CHF are not fully understood. Therefore, we investigated whether there is a role for CIF in this pathologic condition.**

Methods. **Plasma and urinary CIF levels were investigated in 23 patients with chronic CHF and compared with changes in plasma atrial natriuretic peptide (ANP). Twelve patients without CHF served as control subjects.**

Results. **CHF was associated with a highly significant threefold increase in both plasma CIF levels (mean ± SD 7.10 ± 3.01 vs. 2.28 ± 0.92 U/ml, $p < 0.0001$) and urinary CIF excretion (7,849 ± 3,600 vs. 2,351 ± 1,297 U/day, $p < 0.0001$) with respect to patients without CHF. CIF increased as a function of impairment in left ventricular ejection fraction ($r = -0.703$, $p < 0.0001$) and the severity of clinical status. Plasma ANP was also increased in patients with CHF, although to a lesser extent (68%, $p = 0.0501$) than plasma CIF, and was also significantly correlated with left ventricular ejection fraction ($r = -0.552$, $p = 0.0004$).**

Conclusions. **Plasma and urinary CIF activities were strongly and very significantly increased in chronic CHF. In addition to ANP, this long-term natriuretic agent may be of potential importance in reducing fluid overload in CHF.**

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Volume overload, a major disorder of congestive heart failure (CHF), provokes important adjustments in fluid volume regulatory mechanisms. Thus, plasma levels of atrial natriuretic peptide (ANP) are elevated in CHF (1,2), and brain natriuretic peptide (BNP), which has longer kinetic activity, seems to play an important role as a counterregulatory cardiac hormone (3). Moreover, other, not fully characterized natriuretic mechanisms may play a role in CHF.

We previously described in salt-loaded rats, a new endogenous natriuretic compound acting like loop diuretic drugs, that is, potently inhibiting the furosemide-sensitive sodium-potassium-chloride (Na-K-Cl) cotransport system (cotransport inhibitory factor (CIF) (4–7). Extensive work in the purification and chemical analysis of CIF is being conducted in our laboratory (5,8). CIF seems to be a steroid compound, chemically related to the nonpeptide, nondigitalis-like natriuretic factor described by Bricker et al. (9) (an isomer of cortisone) and different from the previously characterized endogenous digitalis-like factors: ouabain or a closely related isomer (10,11) and bufodienolide (12).

In this study, we investigated whether CIF plays a role in CHF. Therefore, we measured plasma and urinary CIF in patients with CHF. CIF changes were examined in relation to changes in ANP and severity of the disease.

Methods

Study patients. Twenty-three patients with mild to severe symptoms of CHF (14 men, 9 women) aged 55.7 ± 12.6 years (Table 1) were studied; 10, 7 and 6 subjects were considered to be in New York Heart Association functional class II, III or IV, respectively. Table 2 shows mean values ±SD for the reduction in left ventricular ejection fraction and for left ventricular dilation in patients in these three functional classes. The patients had had symptoms of CHF for >6 months before hospital admission. All but five had sinus rhythm. Detected etiologic factors are listed in Table 1. Left ventricular ejection fraction was determined at the time of the hospital stay by contrast or radionuclide angiography. Patients with severe renal or hepatic failure were excluded. The usual treatment was not modified except for administration of diuretic drugs. It is important to note that 1) except for *loop* diuretic drugs, no other antihypertensive compound listed in Table 1 can inhibit Na-K-Cl cotransport fluxes; 2) 11 patients never received diuretic drugs; 3) in 3 patients, diuretic treatment was stopped 4 days before blood and urine sampling; and 4) in the remaining 9 patients, loop diuretics were replaced by thiazide

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Abbreviations and Acronyms

ANOVA = analysis of variance
ANP = atrial natriuretic peptide
CHF = congestive heart failure
CIF = cotransport inhibitory factor
IC_{50} = 50% inhibition of CIF activity
P_{CIF} = CIF activity in plasma
U_{CIF} = CIF activity in urine
$U_{CIF}V$ = urinary CIF excretion

drugs (compounds that do not interfere with the CIF assay because they are unable to inhibit cotransport fluxes) 4 days before blood and urine sampling. Finally, all patients had a controlled low salt diet with 100 mmol daily sodium intake for 3 days before the study. After withdrawal of blood and urine samples, treatment with loop diuretics and the usual sodium diet were restored if necessary.

The study protocol was approved by the Ethics Committee of our local hospital, and all patients gave a written informed consent.

Patients without CHF. Twelve patients *without CHF*, matched by gender and age with the 23 patients with CHF (Table 1), were included in the study. These patients had no history of cardiac failure, had no symptoms of CHF and had normal angiographic left ventricular ejection fraction. Eight of the 12 were recruited from a group of patients with angina pectoris scheduled for cardiac catheterization. Three had medically controlled hypertension. None was receiving diuretic therapy.

Control subjects. Six normal volunteers matched by gender and age with the other groups (Table 1) served as a control group. These subjects had no history of cardiac disease and had normal cardiac function as assessed by echocardiography. None was receiving medical therapy. In this group, in contrast to the two patient groups, left ventricular ejection fraction was not calculated from isotopic or angiographic analysis but estimated from echographic analysis. Consequently, we did not use these ejection fractions for the statistical analysis.

Blood and urine sampling. Subjects were confined to bed for 8 h until blood samples were withdrawn the following morning. Blood samples were obtained by means of an intravenous cannula inserted in an arm vein and flushed with a heparinized saline solution. The initial blood samples were used for hemoglobin, white cell count and biochemistry determinations (sodium, potassium, chloride, urea, creatinine). For ANP determination, a blood sample of 5 ml was collected in a tube containing ethylenediaminetetraacetic acid (EDTA) and aprotinin (1 million IU/ml). For CIF activity, 10 ml of blood was collected in a plastic tube. Blood samples for ANP and CIF activity determinations were placed in the cold and immediately centrifuged at 1,750 *g* for 15 min at 4°C. The supernatant plasma was removed and stored at −80°C.

Urine samples were obtained from a 24-h urine collection. One sample was processed for the measurement of urea, creatinine, sodium, potassium and chloride. For measurement of CIF activity, a second sample was centrifuged at 5,000 *g* for 10 min at 4°C, and the supernatant was removed and stored at −80°C.

Measurement of ANP. ANP contents in plasma were measured by using a previously described radioimmunoassay technique (13). Briefly, plasma ANP was extracted with Vycor glass (Corning Glassware). Absorbed ANP was eluted with water-acetone and dried. The pellet was reconstituted in saline solution and added to rabbit anti-alpha-atrial natriuretic polypeptide serum and the mixture was incubated at 4°C for 24 h. The next day, iodine-125-ANP (Amersham Labs) was added and the mixtures were incubated for an additional 24 h. The radiolabel was separated by addition of dextran and coated charcoal solution followed by centrifugation. Values were expressed in pg/ml.

Measurement of CIF activity. For each subject, plasma and urine samples were diluted in saline solution and tested in concentration-response curves for their inhibitory potency on membrane fluxes catalyzed by the Na-K-Cl cotransport system (4–7). Na-K-Cl cotransport fluxes were measured in human erythrocytes accordingly to a previously described protocol (4–7). Briefly, fresh human erythrocytes were loaded with lithium (Li) by using the nystatin ionophore (14). Li-loaded cells were incubated in Li-free media and Li^+ efflux was measured by atomic absorption (14). Na-K-Cl cotransport activity was equated to the bumetanide-sensitive Li^+ efflux (for technical details see ref. 4–7,14).

For each subject, the percent sample dilution, vol/vol, required to obtain 50% inhibition of cotransport activity (IC_{50}) was calculated from the dose-response curves. CIF activity in plasma (P_{CIF}) or urine (U_{CIF}) was calculated as a percent of $1/IC_{50}$ and expressed in U/ml. Finally, measurement of daily diuresis allowed the calculation of urinary CIF excretion ($U_{CIF}V$, see ref. 6,7 for details).

Statistical methods. All results in figures, tables and text are reported as mean value ± SD. Statistical differences between mean values were determined by using an unpaired Student *t* test. Correlations were obtained by linear regression analysis. Multiple measurement comparison was performed by using an analysis of variance (ANOVA) program followed by a Bonferroni/Dunn test. Factorial analysis was also performed by using ANOVA. Statistical significance was accepted for p values < 0.05.

Results

General features. Table 1 shows the general characteristics of the subjects studied. The patients with CHF 1) did not differ significantly in male/female ratio and age with respect to patients without CHF and control subjects, 2) had a strongly reduced left ventricular ejection fraction (34.0 ± 17.0% vs. 63.1 ± 7.6% in patients without CHF, $p < 0.0001$), and 3) did not differ significantly in general biologic variables, except for a small (1.7%) but significant ($p = 0.02$) decrease in plasma sodium levels.

Table 1. Clinical, Biologic and Therapeutic Aspects in the Two Patient Groups and Control Subjects

	Patients With CHF (n = 23)	Patients Without CHF (n = 12)	Control Subjects (n = 6)
Male female	14/9	8/4	4/2
Age (yr)	55.7 ± 12.6	57.2 ± 12.3	58.2 ± 12.0
Weight (kg)	71.9 ± 14.8	72.0 ± 8.8	
Height (cm)	170.8 ± 7.2	173.9 ± 4.3	
Sinus rhythm	18	12	6
LVEF	34.0 ± 17.0%*†	63.1 ± 7.6%	65 ± 6%
Etiology			
Coronary artery disease	13	8	
Valvular disease	4	1	
MI	11	0	
DCM	5	0	
Hypertension	4	3	
Treatments			
Beta-blockers	3	5	
Digoxin	6	0	
Calcium antagonists	2	5	
Nitrates	4	5	
ACE inhibitors	16	3	
Anticoagulants	8	3	
Aspirin	8	8	
Diuretic drugs	12	6	
Plasma			
Na (mmol/liter)	136.6 ± 2.9*	138.9 ± 1.9	
K (mmol/liter)	3.8 ± 0.4	4.1 ± 0.3	
Urea (mmol/liter)	8.3 ± 3.1	6.5 ± 0.5	
Creatinine (μmol/liter)	111.1 ± 28.7	108.8 ± 21.8	
Urine			
Volume (ml/24 h)	1,163 ± 331	1,315 ± 358	
Na (mmol/liter)	56.0 ± 22.2	67.5 ± 33.8	
K (mmol/liter)	49.4 ± 24.6	43.3 ± 22.8	
Urea (mmol/liter)	224 ± 83	238 ± 125	
Creatinine (μmol/liter)	7.85 ± 5.5	10.9 ± 3.5	
Hematocrit	39.3 ± 5.4%	42.4 ± 5.8%	

*,†$p < 0.05$ (by unpaired Student t test) for patients with congestive heart failure (CHF) versus *patients without CHF or †control subjects. Values presented are mean value ± SD. ACE = angiotensin-converting enzyme; DCM = dilated cardiomyopathy; K = potassium; MI = myocardial infarction; Na = sodium.

Atrial natriuretic peptide. Table 3 shows plasma ANP concentration values in the three groups. Mean plasma ANP levels were increased in patients with CHF (by 68% and 150% with respect to patients without CHF and control subjects, respectively). These increased ANP levels in patients with CHF were 1) statistically significant with respect to values in control subjects, and 2) at the limit of the statistical significance ($p = 0.0501$) with respect to patients without CHF. Finally, hypertension and cardiac rhythm were without significant influence on ANP levels (ANOVA factorial analysis; most patients were normotensive and had sinus rhythm).

Mean plasma ANP was 49% higher in patients without CHF than in control subjects, although the difference was not statistically significant (even the nine normotensive patients

Table 2. Angiographic and Echographic Data in the Two Patient Groups

		Patients With CHF		
	Patients Without CHF (n = 12)	NYHA Class II (n = 10)	NYHA Class III (n = 7)	NYHA Class IV (n = 6)
LVEF (%)	63.1 ± 7.6	48.0 ± 15.0*	24.1 ± 8.9*†	22.2 ± 8.5*†
LVEDD (mm)	51.4 ± 3.0	55.5 ± 9.8	61.0 ± 7.1*	64.1 ± 8.4*

*$p < 0.05$ comparing patients with and without congestive heart failure (CHF). †$p < 0.05$ comparing patients with CHF in New York Heart Association (NYHA) functional class II with patients with CHF in functional classes III and IV. Values presented are mean value ± SD. LVEDD = left ventricular end-diastolic diameter; LVEF = left ventricular ejection fraction.

Table 3. Atrial Natriuretic Peptide and Cotransport Inhibitory Factor Levels in the Two Patient Groups and Control Subjects

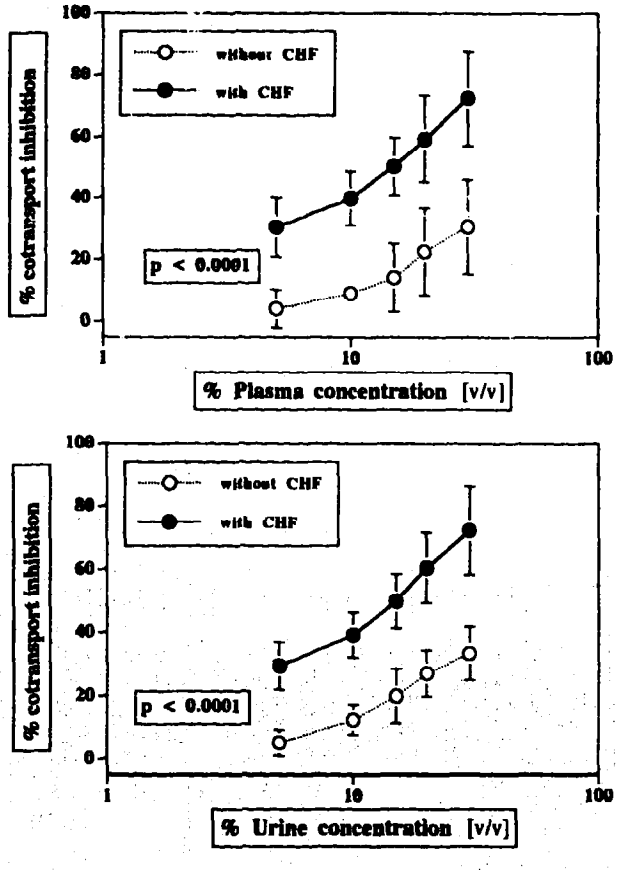
	Patients With CHF (n = 23)	Patients Without CHF (n = 12)	Control Subjects (n = 6)
ANP (pg/ml)	123.6 ± 70.7†	73.3 ± 67.0	49.3 ± 28.6
Plasma CIF (U/ml)	7.10 ± 3.01*†	2.28 ± 0.92†	1.36 ± 0.39
Urinary CIF excretion (U/day)	7,849 ± 3,600*	2,351 ± 1,297	

*$p < 0.05$ comparing patients with and without congestive heart failure (CHF). †$p < 0.05$ comparing patients and control subjects. Values presented are mean value ± SD. ANP = atrial natriuretic peptide; CIF = cotransport inhibitory factor.

without CHF had 69.4% higher ANP levels than those of control subjects).

CIF. For each subject, plasma and urine samples were diluted at different concentrations and tested for their inhibitory activity on cotransport fluxes as described in the Methods section. Figure 1 shows concentration-response curves for cotransport inhibitory potency (mean values ± SD) of plasma (**upper panel**) and urine (**lower panel**). Only the two patient groups (with and without CHF) are represented in this figure. At each dilution, plasma and urine from patients with CHF induced a highly significant ($p < 0.0001$, ANOVA multiple measurement comparison followed by Bonferroni/Dunn test) and more potent cotransport inhibition than did plasma or urine from patients without CHF.

Figure 1. Inhibition of sodium-potassium-chloride (Na-K-Cl) cotransport fluxes by plasma (**upper panel**) and urine (**lower panel**) from patients with chronic congestive heart failure (CHF). Values presented are mean value ± SD. Statistical significance was calculated by using analysis of variance multiple measurement comparison followed by the Bonferroni/Dunn test. v = volume.



For each subject, the plasma or urine dilution required to achieve IC_{50} was calculated from individual curves similar to that of Figure 1 (see Methods). Plasma from patients with CHF had 2.9 times lower IC_{50} for cotransport inhibition than that of patients without CHF (IC_{50} 17.0 ± 9.2% vs. 49.2 ± 16.1%, $p < 0.0001$). This decrease in IC_{50} was still greater (4.6 times) when patients with CHF were compared with the healthy control subjects (IC_{50} 77.7 ± 19.2%). Moreover, it was independent of diuretic treatment; that is, patients untreated with diuretic drugs had an IC_{50} (16.1 ± 3.5%, n = 11) similar to that of patients whose treatment with loop diuretics was interrupted before the study (18.0 ± 1.7%, n = 12). It is important to mention that 1) interassay and intraassay variations in IC_{50} values were 13.4% and 4.3%, respectively (SD/mean in %, n = 6), and 2) in the healthy control subjects, IC_{50} was modestly (58%) but significantly ($p = 0.0042$) higher than in the patients without CHF.

Similar results were obtained with urine samples. Thus, patients with CHF had a 3.7 times lower IC_{50} for cotransport inhibition than did patients without CHF (IC_{50} 17.5 ± 8.3% vs. 65.1 ± 22.9%, respectively, $p < 0.0001$), and this lower IC_{50} was also independent of diuretic treatment.

For each subject, plasma CIF activity (P_{CIF}) and urinary CIF excretion ($U_{CIF}V$) were calculated from the individual values of IC_{50} and daily diuresis as described in the Methods section. Table 3 shows that patients with CHF had a highly significant ($p < 0.0001$) threefold increase in both P_{CIF} and $U_{CIF}V$ with respect to values in patients without CHF. The increase in P_{CIF} was still higher (5.2 times) when patients with CHF were compared with healthy control subjects (Table 3, $p < 0.0001$). Hypertension and cardiac rhythm were without significant influence on both P_{CIF} and $U_{CIF}V$ (most patients were normotensive and had sinus rhythm).

To investigate the influence of CHF etiology, patients with CHF who had coronary artery disease, normal blood pressure and sinus rhythm were compared with patients with CHF without coronary disease. Table 4 shows no significant differences between these two subgroups in plasma ANP or CIF levels or urinary CIF excretion.

Patients without CHF showed a modest but significant increase in P_{CIF} (68%, $p = 0.033$) with respect to values in control subjects (Table 3), and even the nine normotensive

Table 4. Plasma and Urinary Cotransport Inhibitory Factor in Patients With Congestive Heart Failure With and Without Coronary Artery Disease*

	CHF With Coronary Artery Disease (n = 9)	CHF Without Coronary Artery Disease (n = 11)
ANP (pg/ml)	142.7 ± 85.5	111.0 ± 65.8
Plasma CIF (U/ml)	6.78 ± 1.58	8.25 ± 3.55
Urinary CIF excretion (U/day)	8,239 ± 3,462	8,032 ± 3,891
LVEF (%)	33.7 ± 13.6	36.6 ± 22.6

*Cotransport inhibitory factor (CIF) values in patients with congestive heart failure (CHF) and coronary artery disease, normal blood pressure and sinus rhythm were compared with those in patients with CHF without coronary artery disease and with other etiologic factors for CHF. There were no significant differences between these subgroups. Values presented are mean value ± SD. Other abbreviations as in Tables 2 and 3.

patients without CHF had P_{CIF} levels significantly higher (77.3%, p = 0.027) than those of control subjects.

P_{CIF} and $U_{CIF}V$ values were strongly correlated (r = 0.79, p < 0.0001) in the total study group. This correlation remained statistically significant (r = 0.597, p = 0.0027) in the group of patients with CHF alone. P_{CIF} values were also significantly correlated with plasma ANP values (r = 0.376, p = 0.0148) in the total study group. However, the correlation was not statistically significant in patients with CHF alone (r = 0.295, p = 0.0859).

ANP and CIF levels as a function of severity of disease. The inclusion of patients without CHF, whose left ventricular ejection fraction was measured angiographically, allowed us to investigate the influence of this variable. Figure 2 shows that both plasma CIF and plasma ANP were significantly correlated with ejection fraction (for CIF, r = −0.703, p < 0.0001; for ANP, r = −0.552, p = 0.0004).

Urinary CIF excretion was also strongly correlated (r = −0.675, p < 0.0001) with left ventricular ejection fraction. All correlations persisted when only patients with CHF were considered: plasma CIF, r = −0.492, p = 0.019 and urinary CIF excretion, r = −0.428, p = 0.046. Figure 3 shows that plasma CIF levels tended to be significantly higher in patients with poor clinical status (p < 0.05 for functional class IV vs. class II).

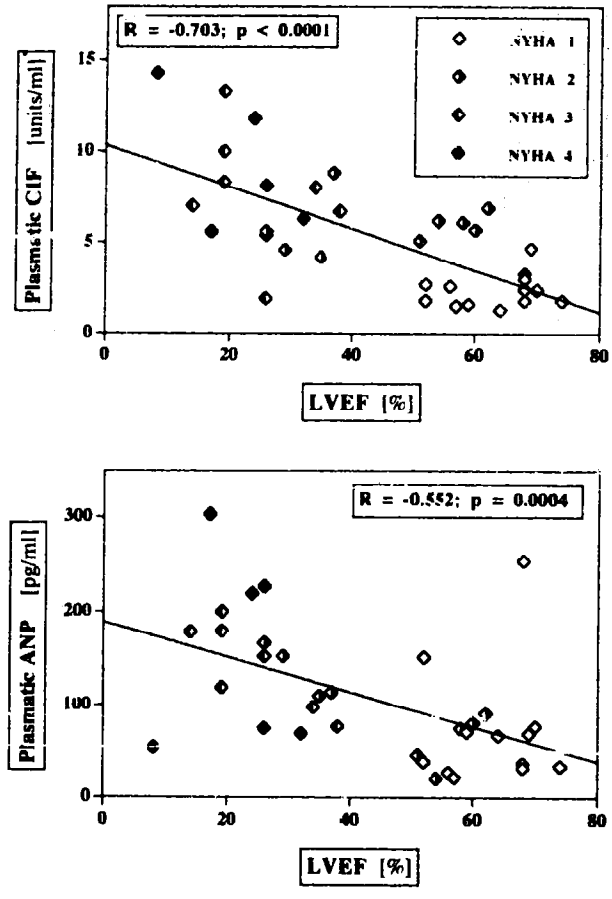


Figure 2. Plasma cotransport inhibitory factor (CIF) **(upper panel)** and atrial natriuretic peptide (ANP) **(lower panel)** as a function of left ventricular ejection fraction (LVEF). The impairment in ejection fraction was strongly correlated with the increase in both natriuretic factors. Symbols are the same for both panels. NYHA 1, 2, 3, 4 = New York Heart Association functional classes I, II, III and IV, respectively.

Discussion

Volume overload in CHF is a consequence of the inability of the kidney to excrete sodium (due to the high levels of neurohormones and a decreased renal blood flow). The homeostatic mechanisms regulating sodium excretion by the kidney in normal and pathologic conditions have been investigated for many years. These studies revealed that glomerular filtration rate, renin-angiotensin-aldosterone system, renal nerves and ANP are major regulators of renal sodium excretion. However, there is evidence that other poorly understood mechanisms should play an important role. Thus, several authors suggested the existence of circulating natriuretic factors inhibiting the sodium-potassium pump (or sodium-potassium-adenosine triphosphatase) in the renal tubule (for review see ref. 15). One of these endogenous digitalis-like compounds was reported to possess a chemical structure similar to that of ouabain (10,11), whereas a second one seems to be a bufodienolide (12). However, oral or intravenous cardiac glycosides are unable to produce natriuresis, and the interest in endogenous digitalis-like substances has switched from a role in volume regulation to a role in blood pressure regulation.

CIF is a newly identified neurohumoral natriuretic compound that is different from the endogenous "digitalis-like" substance (it does not inhibit the sodium-potassium pump or sodium-potassium-adenosine triphosphatase, ref. 4–7). This compound, which is undergoing extensive characterization in our laboratory, inhibits the furosemide-sensitive Na-K-Cl cotransport system, a transport system catalyzing sodium chloride reabsorption at the lumen side of the thick ascending limb of Henle's loop.

Preliminary evidence suggesting the existence of CIF came

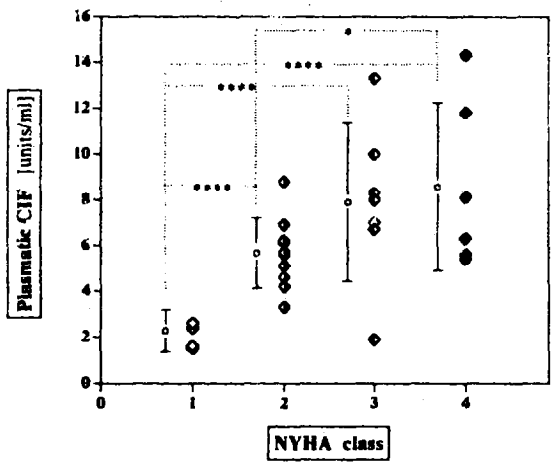


Figure 3. Plasma cotransport inhibitory factor (CIF) levels in patients with congestive heart failure as a function of severity of clinical status. Values presented are mean value ± SD. **** and * indicate $p < 0.0001$ and $p < 0.05$, respectively. NYHA 1, 2, 3, 4 = New York Heart Association functional classes I, II, III and IV, respectively.

from observations in healthy human subjects that oral (16) or intravenous (17) salt loading provokes the inhibition of cotransport fluxes in erythrocytes. Recently, CIF directly detected in urine from salt-loaded rats was found to potently inhibit cotransport fluxes in Madin and Darby canine kidney line (MDCK) cells and human erythrocytes (4). A partially purified urinary fraction enriched in CIF was therefore prepared and intravenously infused in rats (5). This procedure potently increased renal sodium and water excretion for long periods, leaving total urinary potassium unchanged. Recently, we (7) reported that salt-loading in rats induced the appearance of CIF activity in the neurohypophysis. In addition to the different sites of secretion and mechanisms of action of CIF and ANP, one intriguing question is the need for two natriuretic factors. Experiments in rats (6,7) revealed that CIF and ANP have very different although complementary kinetic behaviors. Thus, long-term salt-loading in rats induced 1) a rapid and transient increase in plasma ANP levels (with a peak within the 1st 24 h), and 2) a delayed urinary CIF excretion, that increased slowly, reaching stationary levels after 4 to 6 days of salt loading (6,7). Taken together, these results show that 1) CIF is a newly identified neurohumoral natriuretic factor with a part but not all of the biologic profile of loop diuretic drugs, and 2) in contrast to ANP, CIF seems to regulate *long-term* renal sodium excretion.

Several observations and assumptions suggested to us that CIF could be increased and play an important compensatory role in CHF. First, ANP (1,2) and endogenous ouabain (18) are increased in CHF, and CIF tends often to change in parallel with these two factors. Second, we considered it likely that a long-term natriuretic factor would regulate chronic volume overload. Finally, CIF has a profile similar to that of loop diuretic drugs, which are useful in treating volume overload in CHF.

The present study revealed important increases in plasma and urinary CIF activity in patients with CHF. ANP or the "digitalis-like" compound cannot account for such results, because 1) neither compound is able to inhibit Na-K-Cl cotransport in human red cells (19), and 2) purified CIF preparations were free from immunoreactive ANP and digoxin activity (5). Moreover, an interference of diuretic treatment was excluded because only loop diuretics can inhibit erythrocyte cotransport. CIF was increased to a similar extent in patients who never received diuretic drugs and the increase in CIF was independent of previous diuretic treatment.

Increases in plasma CIF were twice as great as those of plasma ANP and were very significantly correlated with the decrease in left ventricular ejection fraction ($r = -0.703$, $p < 0.0001$). Urinary CIF excretion was also increased in patients with CHF and was also highly significantly correlated with the decrease in ejection fraction.

To investigate the influence of left ventricular ejection fraction, we also studied patients without CHF. This group, which included patients with hypertension and coronary artery disease, showed a modest (68%) but significant ($p = 0.033$) increase in P_{CIF} with respect to values in healthy control subjects. Therefore, 1) patients with CHF had still higher and even more significant increases in plasma CIF activity with respect to values in healthy control subjects than with respect to values in patients without CHF (Table 3), and 2) other cardiovascular abnormalities should also increase CIF, although to a much lower extent than the increase induced by CHF.

Our results suggest that CIF can be a new biochemical marker of CHF. As such, CIF has the advantage of remaining stable in cold temperatures. The assay of cotransport fluxes is very sensitive and precise but not easily adapted to large scale CIF quantification. Therefore, an effort should be made to develop radioimmunoassays allowing large scale CIF measurements. This step requires further clinical studies and further chemical characterization of CIF.

Limitations of the study. The main limitation of this study is that cotransport inhibition in patients with CHF was studied with use of a biologic assay without providing chemical characterization of CIF. Indeed, such chemical work was initiated in our laboratory as soon as bioassays revealed the presence of large amounts of CIF in urine from salt-loaded rats (4). That investigation revealed that rat urine has at least two compounds with CIF activity: 1) equol (3,4-dihydro-3-(4-hydroxyphenyl)-2H-1-benzopyran-7-ol), an "estrogen-like" isoflavonoid that is synthesized by intestinal bacteria and explains basal urinary CIF activity, and 2) a retropituitary CIF compound that is evoked by salt loading (8). This latter compound, a very potent cotransport inhibitor was present in lower concentrations than equol; we hope to report on its structure in the near future. Human urine contains much lower amounts of equol (<0.1 μmol/liter) than does rat urine (~20 μmol/liter [8]). Therefore, it seems unlikely that equol interfered with the measurement of CIF activity in our patients with CHF.

Similarly, the demonstration of cotransport inhibition

should be done at the Henle's loop level. Finally, nothing is known concerning the regulatory mechanisms of CIF secretion by the neurohypophysis. Despite such limitations, this study is the first to demonstrate the presence of an endogenous natriuretic factor other than ANP in CHF, where it can have an important regulatory role.

Another limitation was the small size of the study group and the heterogeneity of patients with CHF. However, within the homogeneous group of patients with ischemic cardiomyopathies, CIF values were not different from those found for the total study group. Moreover, few patients were hypertensive or without sinus rhythm, and these factors did not significantly influence CIF levels. Therefore, CHF seems to be a main factor increasing CIF levels, independent of etiology and other potentially confounding factors.

Clinical implications. The present study suggests that in addition to changes in ANP, changes in CIF in patients with CHF can express the triggering of homeostatic, long-term mechanisms to counterbalance the sodium and water retention that characterizes this disease. The presence of an endogenous compound that acts like furosemide suggests that different levels of natriuresis could be regulated by CIF and that the pathologic consequences of fluid retention may be due to abnormal CIF regulation. This hypothesis merits confirmation by further studies. Such studies can open up an attractive new field of research that may improve our understanding of CHF, although whether CIF research will lead to different therapeutic approaches to CHF is speculative.

In conclusion, plasma and urinary CIF activity was strongly and very significantly increased in patients with CHF. Increases in CIF were very significantly correlated with the decrease in left ventricular ejection fraction. These changes in CIF are of potential importance as they can express the triggering of homeostatic, long-term mechanisms to counterbalance fluid overload in CHF. Other studies are required to further characterize the chemical structure of CIF and its role in CHF.

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